Title: Single dose liposomal amphotericin for asymptomatic cryptococcal antigenemia (ACACIA)

Grant title: Evaluation of CrAg screening with enhanced antifungal therapy for asymptomatic CrAg+ persons

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with Good Clinical Practice (GCP) as required by the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312)
- ICH E6; 62 Federal Register 25691 (1997)
- Uganda National Council of Science and Technology (UNCST)

All key personnel (all individuals responsible for the design and conduct of this study) have completed Human Subjects Protection Training.

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CRAG	Cryptococcal Antigen
CRF	Case Report Form
CSF	Cerebrospinal fluid
DSMB	Data and Safety Monitoring Board
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
IND	Investigational New Drug Application
IRB	Institutional Review Board
MIC	Minimum Inhibitory Concentration
MOP	Manual of Procedures
N	Number (typically refers to subjects)
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure
US	United States
WHO	World Health Organization

PROTOCOL SUMMARY

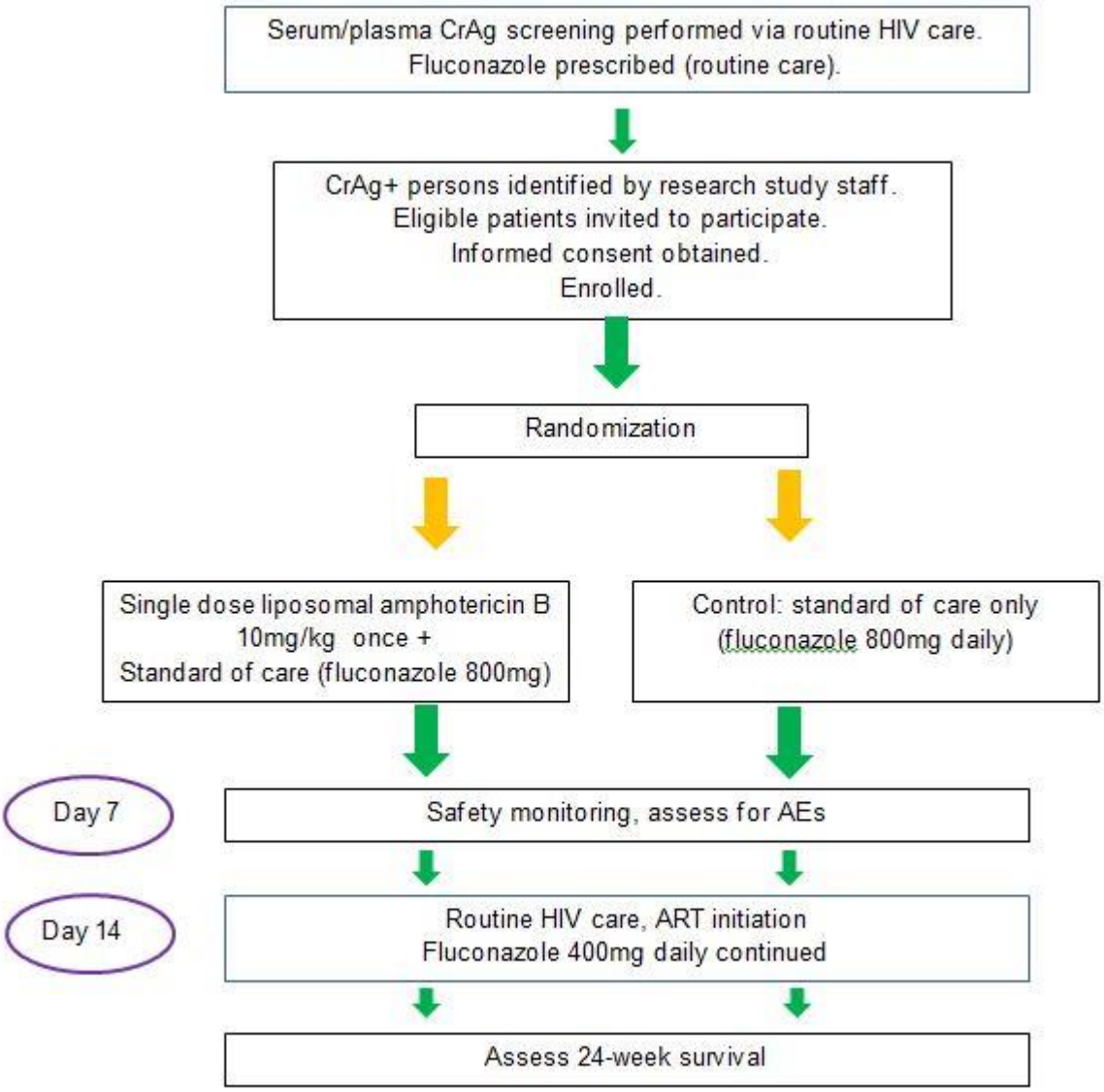
Title	Single dose liposomal amphotericin for asymptomatic cryptococcal antigenemia
Grant Title	Evaluation of CrAg screening with enhanced antifungal therapy for asymptomatic CrAg-positive persons
Population	HIV-infected persons with asymptomatic cryptococcal antigenemia in Uganda
Study Design	Randomized controlled trial
Study Phase	Integrated Phase II / III
Study Setting	Uganda
Study Duration	Stage 1 (Phase II trial): 1 year Stage 2 (Phase III trial): ~4 years in total
Subject Duration	24 weeks
Description of Intervention	Single dose liposomal amphotericin (AmBisome) at 10mg/kg intravenous
Standard of Care	Fluconazole 800mg daily x 2 weeks followed by 400mg daily x 8 weeks, then 200mg/day secondary prophylaxis
Hypothesis	With standard of care, the meningitis-free survival is ~75% in CrAg+ persons compared to ~90% in CrAg-negative persons with CD4<100 cells/μL. Persons with higher CrAg titers in blood are at higher risk of death, thus we hypothesize *Cryptococcus* is responsible even in the absence of overt "meningitis" representing a spectrum of disseminated cryptococcosis causing sepsis, pneumonitis, and encephalitis. We hypothesize enhanced antifungal preemptive therapy will improve survival in CrAg+ persons.
Rationale	Liposomal amphotericin B dosed once at 10mg/kg is routinely used for visceral leishmaniasis therapy and as weekly prophylaxis in stem-cell transplant patients. The 10mg/kg dose is safe with zero of 304 participants having any residual nephrotoxicity at 30 days in a visceral leishmaniasis trial.[1] In a small phase II cryptococcal meningitis trial (n=80), single 10mg/kg dose was non-inferior compared to 2-weeks of amphotericin B with trends toward better survival, faster CSF clearance, and less toxicity.[2]

Equipoise **(Safety vs. Efficacy)**	The fundamental risk consideration is the balance between drug-related toxicity versus 25-30% meningitis and/or death of CrAg+ persons.
Primary Objective	To determine if 24-week cryptococcal meningitis-free survival is improved with liposomal amphotericin in the treatment of asymptomatic cryptococcal antigenemia in addition to standard of care fluconazole therapy.
Secondary Objectives	1. To determine safety and tolerability of single-dose liposomal amphotericin. 2. To evaluate 24-week survival 3. To evaluate cost-effectiveness of single dose liposomal amphotericin for the treatment of asymptomatic cryptococcal antigenemia
Primary Endpoint	1. 24-week cryptococcal meningitis-free survival Comparison will be made between those randomized to single dose liposomal amphotericin + standard of care (fluconazole) vs. standard of care (fluconazole) alone.
Secondary Endpoints	1. Incidence of grade 3 to 5 clinical adverse events or serious adverse events within two weeks of enrollment 2. Incidence of grade 3 to 5 laboratory adverse events by NIAID DAIDS toxicity scale within two weeks of enrollment 3. 24-week known survival 4. Cost and Incremental cost-effectiveness ratio (ICER) of single dose liposomal amphotericin + fluconazole compared to fluconazole preemptive therapy alone, and compared to no preemptive therapy.
Stage 1	The first 100 persons enrolled will receive intensive safety monitoring. The purpose of this stage is to thoroughly evaluate safety and tolerability of the one-time liposomal amphotericin in asymptomatic CrAg+ persons.
Stage 1 Review "Go / No Go Decision"	The DSMB will review adverse events and interim outcomes after the first 100 persons are enrolled. If deemed to be safe, tolerable, and with favorable efficacy trend, we will proceed to Stage 2.

Stage 2	Up to an additional 500 persons will be enrolled in stage 2. The total sample size will ultimately be dependent on the effect size observed. If AmBisome-related AEs are ≤6% (3/50) in stage 1, scheduled lab monitoring after liposomal amphotericin will be reduced in stage 2. The purpose of stage 2 is to evaluate survival efficacy of liposomal amphotericin. Six-month outcomes will be evaluated for all persons in stages 1 and 2.
A priori Subgroups	• CrAg lateral flow assay (Immy) blood titer >=1:160 vs. <=1:80 • ART-naïve vs. ART experienced • Rifampin use • Symptomatic antigenemia
Description of Study Design	This will be a randomized controlled trial of asymptomatic CrAg + persons in Uganda. They will be randomized to receive preemptive treatment with 1 dose of liposomal amphotericin (10mg/kg) in addition to standard of care fluconazole therapy. We will evaluate 24-week meningitis-free survival and overall survival in those who receive the intervention compared with participants receiving fluconazole per WHO and national standard of care therapy. We will also evaluate survival by CrAg titer (comparing high titer who received AmBisome to high titer who received fluconazole alone), safety, tolerability, and cost-effectiveness of this regimen.
Number of participants to be studied	Up to 600 participants (300 in each arm)
Statistical Assumptions	Control group = 75% meningitis-free survival Intervention group 80% power to detect: • If Survival ~90%, sample size, n=200 in total • If Survival ~87%, sample size, n=300 in total • If Survival ~86%, sample size, n=400 in total • If Survival ~85%, sample size, n=500 in total • If Survival ~84%, sample size, n=600 in total

	For early stopping, a modified Haybittle–Peto boundary using a p-value threshold of P=0.01 will be used. Interim analyses will occur ~100 participants enrolled.

Study Schematic



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1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Cryptococcus neoformans is a major opportunistic pathogen and a leading cause of death among AIDS patients in Sub-Saharan Africa.[3-8] *C. neoformans* is the most common cause of meningitis among adults in Africa, more common than bacterial meningitis.[9] Across the developed world, introduction of antiretroviral therapy (ART) has been associated with a decline in the incidence of cryptococcal meningitis.[10] However, despite major efforts to expand ART access over recent years,[11] in many resource-limited settings CD4+ T-cell (CD4) counts of persons entering HIV care remain low,[12,13] and there is a high risk of new AIDS events and early mortality. In Uganda, CDC data suggest that 16% of persons presenting to care have a CD4<100 cells/μL. In Botswana the incidence of cryptococcal meningitis from 2013 to 2014 is comparable to pre-ART era rates in South Africa, despite excellent ART coverage.[14] Between 8%-26% of patients die during the first year of ART, with most deaths occurring during the first few months of ART.[12,15] Cryptococcal disease remains a leading contributor to this early ART mortality throughout Sub-Saharan Africa,[15-19] and thus prevention efforts should be a priority. **Cryptococcosis accounted for 13- 18% of all deaths in 4 cohorts of HIV-infected persons from Uganda** [4,5,15,20] and **44% of all deaths in a South African cohort**.[6] While the incidence of TB is higher, the mortality burden due to cryptococcosis may approach that of tuberculosis.[3,6,12,21]

Cryptococcal Disease Prevention

One strategy to prevent the early mortality in ART programs is to screen asymptomatic persons for subclinical cryptococcosis using CrAg testing in the blood, and then preemptively treat those with subclinical disease with fluconazole.

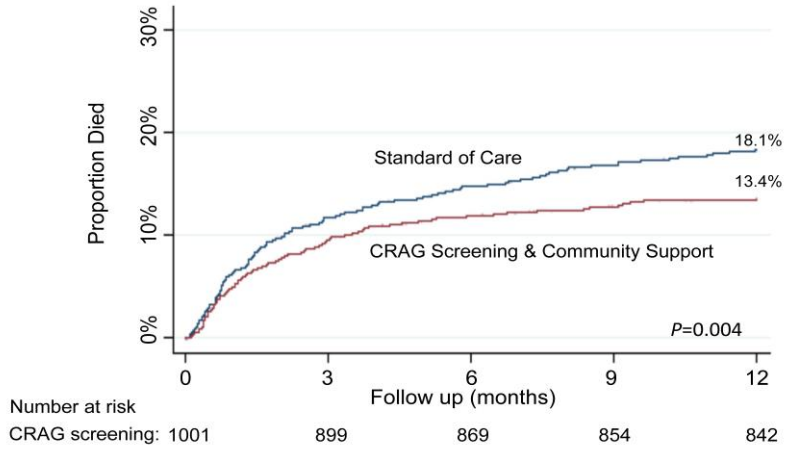
In 2011, and again in March 2018, the WHO recommended screening for CrAg followed by preemptive antifungal therapy for CrAg positive persons, to prevent the development of invasive cryptococcal disease for persons with a CD4 cell count < 100 cells/μL (strong recommendation).[22]

WHO recommended pre-emptive therapy: 800 mg fluconazole daily for 2 weeks, followed by 400 mg daily for 8 weeks and then 200 mg daily until immune reconstitution. This was adopted by the Ugandan national HIV Guidelines.

The WHO acknowledged in the guidelines that the optimal preemptive treatment of CrAg+ persons is not known. This recommended therapy has a 25-30% failure rate with meningitis or death.

This CrAg screen-and-treat strategy has proven to be efficacious in one published randomized clinical trial and two cohorts in Tanzania and Zambia.[23-25] In the trial, CrAg screening and 4 weeks of ART adherence support resulted in a 28% relative reduction in mortality among persons with a CD4 count <200 cells/µL **(Figure 2)**.

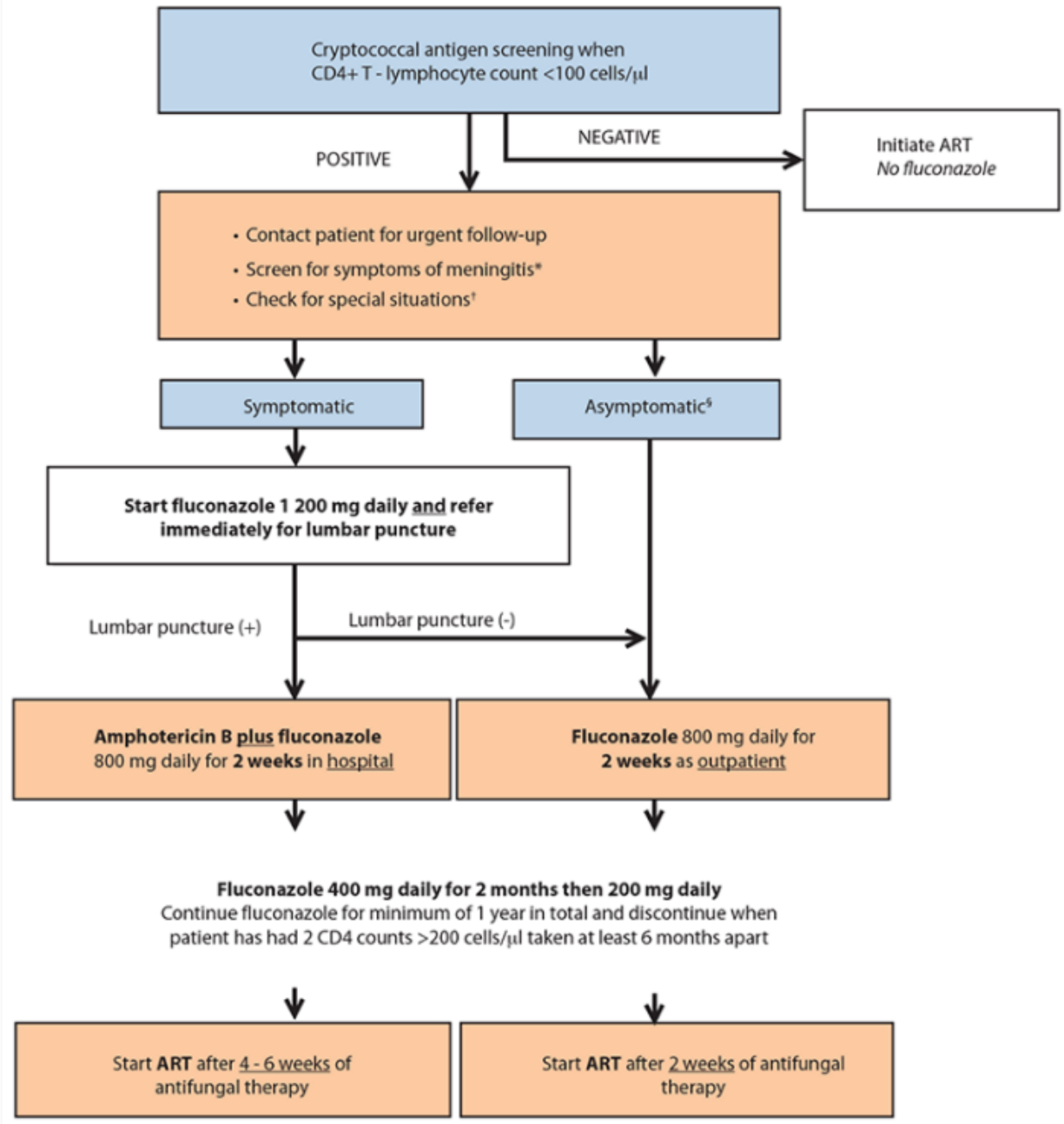
Figure 2. CrAg screening improves survival



Based on South African outcome and cost data, Jarvis *et al* estimated that CrAg screening saves $156 (95%CI: $119 to $197) for every person with a CD4 count <100/µL,[26] and this results in 40%-80% better 5-year survival.[24,27] In Cape Town, 73% of ART-naïve patients presenting with cryptococcal meningitis had already been diagnosed with HIV, a median of 4 months earlier.[28] CrAg screening could identify these patients, allowing for pre-emptive antifungal therapy and fast-tracking for ART initiation – an issue of particular priority, given the exceptionally high mortality of South African patients in this pre-ART initiation period.[28-30] If all patients who previously tested HIV-positive (both those on ART and the 73% who were known to be HIV-infected but not on ART) had been CrAg screened and subsequently preemptively treated if CrAg +, up to 78% of cases of cryptococcal meningitis could have been averted, with significant numbers of lives saved and hospitalization costs averted.

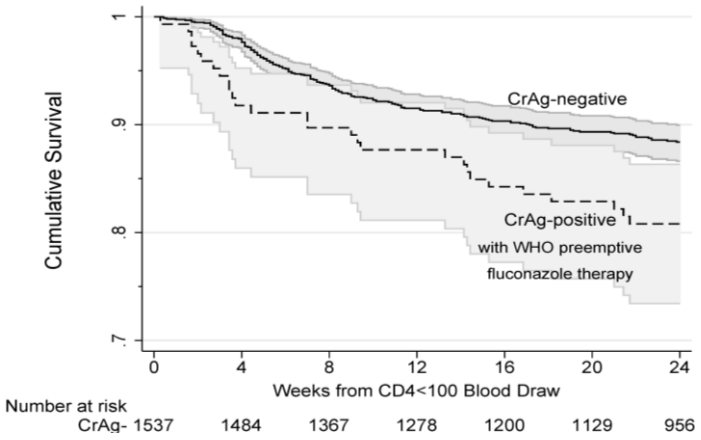
Similar studies in Uganda have found CrAg screening to be highly cost-effective in Uganda, associated with an ICER of $6 per DALY averted compared to no screening.

Due to the overwhelming cost-benefit of this relatively simple intervention, Uganda has included CrAg screening in 2016 national HIV guidelines. One example of a screening algorithm for CrAg screening and treatment is included below:



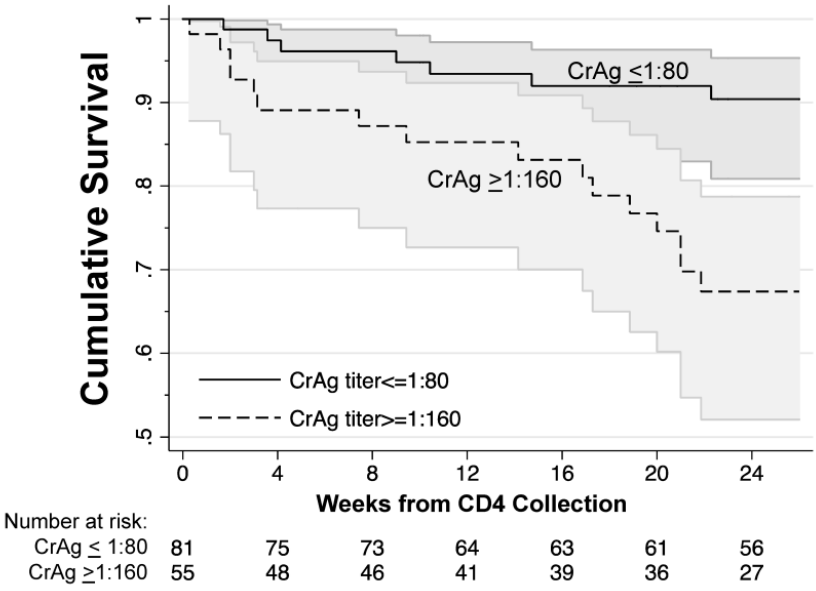
1) Although CrAg+ persons receiving fluconazole generally do better than if not treated at all, 25%-30% go on to develop meningitis or die despite the WHO-recommended antifungal therapy and ART. A 2.9-fold worse survival was observed in asymptomatic CrAg+ vs. CrAg- persons with CD4 count <200 cells/μL in Tanzania and Zambia [31] **(Figure 3)**.

Figure 3. Survival by CrAg status in Uganda & REMSTART trial in Tanzania & Zambia



The current WHO regimen for asymptomatic CrAg+ patients is based on expert opinion and extrapolated from management of symptomatic cryptococcal meningitis. This regimen likely requires further refinement. As an example, preliminary results from the ORCAS trial in Uganda suggest that persons with CrAg titres ≥1:160 and CD4 count <50 cells/μL are more likely to develop subsequent cryptococcal meningitis compared with those with a lower CrAg titre/ higher CD4 count despite receiving current WHO-recommended antifungal therapy. Customized or more intensive therapy for those with high titres may be a useful strategy to reduce CrAg+ mortality **(Figure 4)**.

Figure 4. Survival stratified by CrAg titer in Ugandan ORCAS trial

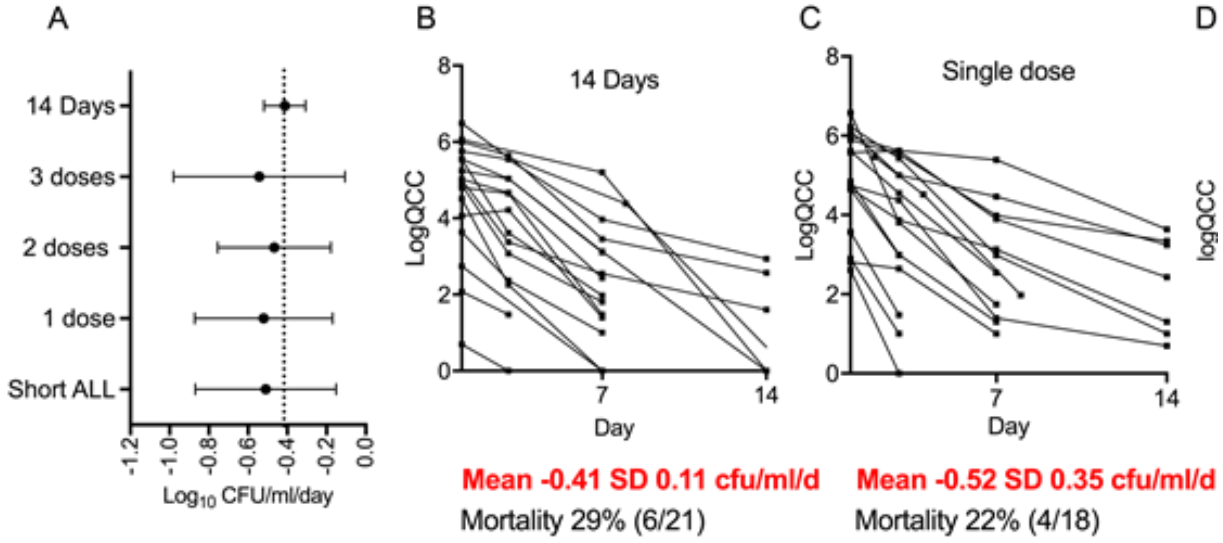


1.2 Liposomal Amphotericin

Liposomal amphotericin is recommended for the treatment of cryptococcal meningitis per IDSA guidelines.[32] U.S. Department of Health and Human Services guidelines (AIDSInfo.gov) recommend amphotericin for treating non-CNS, extrapulmonary cryptococcosis.[33] Amphotericin is likely to be effective in asymptomatic cryptococcal antigenemia, given the lower burden but disseminated nature of infection. However, it is not feasible to hospitalize otherwise asymptomatic patients for 14 days of a medication that causes nephrotoxicity, life-threatening electrolyte abnormalities, phlebitis, and anemia. Hospitalization requires intravenous (IV) access, daily IV fluids and electrolyte supplementation, and rapid, reliable laboratory monitoring. Short course amphotericin (5 to 7 days) has shown no slowing of the rate of clearance of meningitis compared to a full 14-day course.[34] Animal models suggest that regimens as short as 3 days are as effective as a 14-day course.[35]

Liposomal amphotericin may be an optimal treatment regimen for asymptomatic cryptococcal antigenemia, given lower rates of nephrotoxicity and lower rates of infusion reactions compared to traditional amphotericin deoxycholate. Also liposomal amphotericin has a long tissue half-life, and good tissue penetration.[36] One-time high dose liposomal amphotericin of 15mg/kg has been used safely, and 10mg/kg is routinely efficacious in visceral leishmaniasis and has proven to be feasible to administer as an outpatient in resource-limited settings.[37,38]

In a completed phase II trial in cryptococcal meningitis,[37] CSF clearance rate of Cryptococcus yeasts was non-inferior with single 10 mg/kg liposomal amphotericin dose as to 14 days of 3 mg/kg/day traditional liposomal amphotericin therapy (**Figure 5)**.[2]



Mortality amongst those receiving single dose liposomal amphotericin is 22% (4/18), whereas mortality in those receiving 14 days of liposomal amphotericin is 29% (6/21).[2] Single dose liposomal amphotericin is being further evaluated for the treatment of cryptococcal meningitis as the Phase III AMBITION Trial ongoing in Botswana, Malawi, Cape Town, and Kampala &

Mbarara, Uganda.[37] Given liposomal amphotericin initial promise for the treatment of fulminant meningitis, for this study we will use one 10mg/kg dose of AmBisome in addition to fluconazole. A single dose can be administered to an outpatient, asymptomatic population who do not otherwise require hospitalization.

1.3 Clinical Rationale

Despite the commendable effort of rolling out national reflex CRAG screening in laboratories, we anticipate 25 to 30% to develop meningitis or die despite standard of care fluconazole preemptive therapy. The current WHO regimen for asymptomatic CRAG+ patients is based on expert opinion and extrapolated from management of symptomatic cryptococcal meningitis. It is likely that high dose fluconazole is insufficient for the treatment of asymptomatic cryptococcal antigenemia, particularly in those with high titers $\geq$1:160.[39]

The treatment regimen for asymptomatic CrAg+ persons requires further refinement to optimize survival. We seek to administer 1 dose of liposomal amphotericin at 10mg/kg in addition to current standard of care to evaluate efficacy and safety of this more potent regimen. A single dose can be administered to this outpatient, asymptomatic population who do not otherwise require hospitalization, thus this is a potentially implementable strategy.

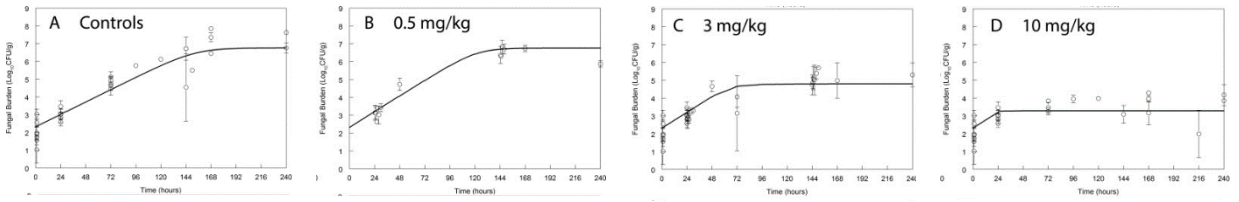
The concept of single or intermittent dosing of liposomal amphotericin is well established. Single liposomal amphotericin doses of up to 15mg/kg have been safely given,[40] and liposomal amphotericin doses of 10mg/kg are routinely given for visceral leishmaniasis;[1] as well as in antifungal prophylaxis for stem-cell transplant patients[40,41] Even at high doses, liposomal amphotericin has significantly less nephrotoxicity, less anemia, and lower rates of infusion reactions than conventional amphotericin B deoxycholate.[2,42]

1.4 Pharmacologic Rationale

It is well known that amphotericin B has a very prolonged tissue half-life. [40,41,43,44] The terminal tissue half-life is 5.5 days (133 hours).[45,46] Abbreviated shorter courses of amphotericin B deoxycholate (5-7 days) have less toxicity and equivalent survival as the traditional 14 days of amphotericin B deoxycholate.[34,47] However, with amphotericin B deoxycholate doses above 1 mg/kg generate significant toxicity. Liposomal amphotericin B is different, where single or intermittent 10-15 mg/kg doses have been used. [40,41]

Dr. William Hope's team in Liverpool has investigated liposomal dosing in murine model.[45] They reported that there was a dose-dependent decline in cryptococcal fungal burden in mice brains, with near-maximal effect observed with liposomal amphotericin at 10 to 20 mg/kg/day (**Figure 6)**.[45]

Figure 6. Murine Model Dosing of Liposomal Amphotericin



In mice (which require more mg/kg doses to be equivalent as humans), the pharmacodynamics of a single dose of 20 mg/kg was the same as that of 20 mg/kg/day administered for 2 weeks.[45] In rabbits, 3 daily liposomal amphotericin doses at 5 mg/kg/day in rabbits were required to achieve fungicidal activity in cerebrospinal fluid.[45] In humans, the 10mg/kg dose given once was equivalent at rate of CSF fungal clearance as 14 days of daily dosing (Figure 5 in Section 1.2) and had equivalent 10-week survival (78% with single dose vs. 71% with 14 days of liposomal amphotericin).[2]

1.5 Potential Risks and Benefits

The fundamental risk consideration of this trial is the balance between liposomal amphotericin drug-related toxicity versus 25-30% mortality of CrAg+ participants.

We hypothesize that the survival benefit is overall greater than any liposomal amphotericin-related toxicity which for a single dose is relatively mild, non-life threatening, and transient in duration.

1.5.1 Potential Risks

Liposomal amphotericin B (AmBisome) has lower rates of drug induced toxicities than traditional amphotericin B deoxycholate. [40,41,43,44]

Though liposomal amphotericin at 10mg/kg has been used for cryptococcal meningitis, and for other conditions such as visceral leishmaniasis, it has never previously been used in asymptomatic cryptococcal antigenemia. The medication has multiple side effects, though notably is typically used in multiple doses over a course of weeks. Common side effects include hypokalemia, hypomagnesemia, anemia, nephrotoxicity, nausea, vomiting, diarrhea, and rash. These side effects typically occur during week 2 of medication administration. Given that we are only administering a single dose of the medication, we expect side effects to occur less frequently. However, we will closely monitor for these adverse events, and treat them as appropriate. Chills may be experienced during the infusion, but this can be managed by premedication with paracetamol and/or antihistamine medications. All potential risks should be reversed once the medication is stopped.

In a phase II trial of 80 participants, the side effects of 10mg/kg x1 liposomal amphotericin were did not differ statistically from traditional 14 days of liposomal amphotericin B.[2] In that Botswana and Tanzania trial, there were 10 Grade III AEs and 2 Grade IV AEs attributable to amphotericin. Both Grade IV AEs occurred in the traditional 14 day dosing group.[2] With liposomal amphotericin, the mean change in serum creatinine was +13% (95%CI, -9 to +35%) from baseline to 14 days.[2] In that trial, one subject developed a grade III creatinine rise, defined as >1.8 to <3.5 upper limit of normal or a 50% to 100% rise above baseline.[2] Thus, the safety profile of single dose amphotericin is good.

In this study, we will monitor kidney function closely. Patient will be closely followed to assess for clinical adverse events. The Data monitoring committee (DMC) will monitor this study.

1.5.2 Known Potential Benefits

Subjects may not directly benefit from participating in this study. We hypothesize that will be a survival benefit by accessing a more effective treatment for asymptomatic cryptococcal meningitis. However, although more effective therapy is to be given to the experimental arm, the effect on survival is unproven in this population. If single dose liposomal amphotericin is effective and tolerable, mortality from asymptomatic cryptococcal antigenemia will be reduced by as much as 30% in this population. We expect that if this regimen is effective, this would change national and international guidelines, and thus improve survival in those with asymptomatic cryptococcal infection and prevent meningitis and death.

By participating in the trial, participants will receive more intensive monitoring for their HIV/AIDS and disseminated cryptococcal infection, being promptly referred for expert medical care if they develop symptomatic cryptococcal meningitis.

2 OBJECTIVES

2.1 Study Objectives

Primary Objectives:

1. To assess 24-week efficacy of single dose liposomal amphotericin in the treatment of asymptomatic cryptococcal antigenemia for cryptococcal meningitis-free survival.

Secondary Objectives:

1. To determine safety and tolerability of single-dose liposomal amphotericin for the treatment of asymptomatic cryptococcal infection.
2. To determine 24-week survival in those who receive the intervention compared to those who receive standard of care.
3. To evaluate cost and cost-effectiveness of single dose liposomal amphotericin for the treatment of asymptomatic cryptococcal antigenemia

2.2 Study Outcome Measures

2.2.1 Primary Endpoint

1. 24-week cryptococcal meningitis-free survival

   Comparison will be made between study arms of those who receive single dose liposomal amphotericin + fluconazole vs. those who receive standard of care fluconazole alone.

2.2.2 Secondary Endpoints

1. Incidence of grade 3 to 5 clinical adverse events or serious adverse events within 2 weeks of enrollment
2. Incidence of grade 3 to 5 laboratory adverse events by NIAID DAIDS toxicity scale within 2 weeks of enrollment
3. 24-week known survival.
   a. Death and lost to follow up would be considered therapeutic failures.
4. Cost and Incremental cost-effectiveness ratio (ICER) of single dose liposomal amphotericin + fluconazole compared to fluconazole preemptive therapy alone, and compared to no preemptive therapy.

2.3 Study Design

The study will be a randomized controlled trial of asymptomatic CRAG+ persons. Participants will be randomized to preemptive treatment with 1 dose of liposomal amphotericin (10mg/kg) in addition to standard of care fluconazole vs standard of care fluconazole alone.

We will recruit CRAG+ participants who have already been screened as part of national CRAG screening guidelines. Clinical sites will receive referrals from rural locations, and potential participants will be transported to clinical sites where informed consent, randomization, and administration of liposomal amphotericin will occur.

Stage 1: The first 100 persons enrolled/randomized will receive intensive safety monitoring. The purpose of this stage is to thoroughly evaluate safety and tolerability of liposomal amphotericin in asymptomatic CRAG+ persons. The Data Monitoring Committee will review adverse events and interim outcomes after the first 100 persons are enrolled. If deemed to be safe and tolerable (i.e. ≤6% incidence of any new lab AE), we will proceed to stage 2 of the study.

Stage 2: An additional 500 persons will be enrolled/randomized. Depending on outcomes from stage 1, monitoring may be reduced, if deemed to be unnecessary after the first 100 persons. The purpose of this stage is to evaluate efficacy of liposomal amphotericin. Six-month outcomes will be evaluated for all persons in stages 1 and 2.

We will evaluate 24 week meningitis-free survival in those who receive the intervention compared to those asymptomatic CRAG+ persons who receive fluconazole per standard of care. We will also evaluate survival by CRAG titer (comparing high titer who received AmBisome to high titer who received fluconazole alone).

Study sites: See appendix

Subjects: Eligible participants will be entering HIV care, age ≥18 years, with asymptomatic cryptococcal antigenemia who provide informed consent. These will predominantly be outpatients presenting from HIV clinics.

Those with symptomatic meningitis at baseline will be excluded, along with those ineligible for treatment (contraindications to fluconazole including liver disease or pregnancy). Consent will be obtained from asymptomatic CRAG+ participants who meet inclusion criteria, and are willing to participate.

Clinical outcomes and adverse events will be actively collected for those enrolled. All participants will be followed for 6-month survival and/or development of meningitis.

Cost-effectiveness analysis

Background: Our team has previously reported CRAG screening to be a highly cost-effective intervention in Africa, costing only $21 per disability-adjusted life year (DALY) averted (compared to no screening).[48] This Ugandan estimate in 2010 used the previous, expensive CRAG latex agglutination test ($16). Since that time, the CRAG Lateral Flow Assay has been approved and implemented with a unit cost of ≤$4 (including shipping, taxes, labor, overhead, etc). Our updated analysis in 2012 estimated a cost of ~$28 to detect 1 asymptomatic CRAG+ person in Uganda, and <$40 to save one life, that is ~$2 per DALY saved.[49,50] CRAG screening is "very cost-effective" compared to the Ugandan GDP.

Based on South African cost data, Dr. Joseph Jarvis used mathematical modeling of a hypothetical cohort to estimate that CRAG screening (compared to no screening) saves $156 (95%CI: $119 to $197) for every person with a CD4 count <100 cells/μL in South Africa.[26] In an evaluation of cryptococcal meningitis treatment strategies in Uganda, we concluded that short course of amphotericin was a "very cost-effective" strategy for meningitis as compared to fluconazole alone, and compared to standard 14-day course of amphotericin with fluconazole.

Our proposed package of care of liposomal amphotericin applies these findings; fluconazole alone is insufficient, yet a full course of amphotericin is not only expensive but has many toxicities. Very short course amphotericin may be the optimal treatment regimen for our population.

Overview: We will perform an economic evaluation of single dose liposomal amphotericin, so that the most efficient intervention can readily be assessed and adopted by Ministries of Health. We will develop a decision analytic model amongst HIV-infected outpatients in Uganda, in order to determine the cost and cost-effectiveness of various CRAG screening and treatment strategies, stratified by CRAG titer from the perspective of the health payer.

We hypothesize that despite the higher cost of liposomal amphotericin (international wholesale cost of US$160 per 50mg vial in South Africa), this regimen will be more efficacious and highly cost-effective in asymptomatic CRAG+ persons with high titers. We hypothesize, that while slightly more efficacious in those with low CRAG titers, adding liposomal amphotericin will not be cost-effective in this subgroup compared to WHO standard of care (fluconazole monotherapy). "Highly cost-effective" is defined by the WHO Commission on Macroeconomics and Health as an incremental cost-effectiveness ratio (ICER) of less than 1x the national annual GDP per capita per DALY avoided.[51]

Experimental Plan:

Model Structure: We will develop a decision analytic model to assess the following CRAG screening strategies:

a) No CRAG screening,

b) CRAG screening and treatment per WHO guidelines (fluconazole only),

c) CRAG screen and treatment using liposomal amphotericin for those with high titers, and fluconazole for those with low titers,

d) CRAG screening and treatment using liposomal amphotericin for all CRAG positives, regardless of titer.

The primary outcome of interest will be the ICER of each intervention. Within these 4 treatment strategies, persons have a probability of dying from non-AIDS related deaths, AIDS-related deaths (including TB), and cryptococcal meningitis specifically. Furthermore, in all strategies, risk of loss to follow up (out of care, not starting ART) will be incorporated. Patients will have a probability of being CRAG+ with high titer, CRAG+ with low titer, or CRAG negative. There will be a risk of developing cryptococcal meningitis amongst CRAG+ and CRAG negatives, with or without preemptive therapy.

Model Data: Data for the model will be taken directly from outcomes of our clinical study described in this protocol. Specifically, we will use the results from our primary objective to identify prevalence of CRAG positivity, incidence of cryptococcal meningitis with preemptive antifungal treatment, and treatment efficacy as measured by 24-week survival. Our group has published 5-year long-term survival data for asymptomatic CRAG positive persons to inform extrapolation beyond 6 months.[27] We will include a 3% annual discount rate scaled to the median survival time.

Loss to follow up rate will be taken from our study, but a range will be used in sensitivity analysis drawn from prior published studies, as this critical factor will be relevant outside of clinical trials, in real-world settings. In the model, those lost to follow up will be screened for CRAG but will not receive their results or receive treatment. Thus, they would not receive the survival benefit of the CRAG intervention. Using a range of those lost to follow up will be important as prior studies with large numbers lost to follow up have not demonstrated the full benefit of a CRAG screening program, presumably because asymptomatic CRAG+ people are not treated.[52] Conversely clinical trials and cohorts with minimal loss to follow up have demonstrated significant survival benefit with CRAG screening.[15,23] Incidence of TB will also be taken from findings in our clinical study, but a range will be used to incorporate variation by clinic and region. We will evaluate the WHO recommended strategy (of fluconazole preemptive therapy) using the outcomes of the parent CAST-NET study, where 800 participants will receive fluconazole therapy alone. We will also consider the 'no CRAG screening' strategy by using historical data collected from the participating Ugandan sites from the 2012 ORCAS study.

Costs will be derived from individual patient data and unit costs for fluconazole, CRAG testing (CRAG Lateral Flow Assay cost, lab overhead, lab worker's pay), liposomal amphotericin, IV fluids given with liposomal amphotericin, and healthcare worker resources to administer the IV medication in an outpatient setting. Costs related to treatment of cryptococcal meningitis, including diagnostic and therapeutic lumbar punctures, 14 days of amphotericin B deoxycholate and fluconazole, and 14-day hospitalization have previously been collected by our

group in 2012, and will be updated for this study.[50] Lifetime costs of HIV care and treatment will be obtained from patient data and published literature.

We will compare the costs and DALYs under each CRAG screening intervention. We will eliminate any dominated strategies, which avert fewer DALYs at a greater cost than alternatives. Among non-dominated strategies, we will calculate the incremental costs and effectiveness (in terms of DALYs averted) of each intervention, relative to the previous most effective intervention, and will compare its ICER to the Ugandan per capita GDP. Interventions with ICER<1x GDP per capita will be designated as “highly cost-effective”, while interventions with an ICER of 1-3x GDP per capita will be deemed “cost-effective”, as per WHO definitions. The liposomal amphotericin patent expired in 2016, thus we will also model changes in costs.

Sensitivity Analysis: Parameters such as CRAG prevalence, efficacy of each treatment strategy, loss to follow up, and incidence of cryptococcal meningitis will be varied through plausible ranges, which will be determined from previous CRAG studies.[53-55] Such variation will make our findings more generalizable to broader populations.

Application: Once the decision analytic model has been created, this model can be applied to various settings (both within Uganda, and within greater sub Saharan African region) by changing CRAG prevalence, costs, treatment outcomes, and 6-month survival according to study setting. Thus, this will be a valuable tool to measure the value of CRAG screening and treatment strategies globally.

3 STUDY ENROLLMENT AND WITHDRAWAL

Study population will predominantly be from outpatient clinics. Those identified as CRAG+ by lab test will typically be HIV-infected outpatients presenting for routine care.

3.1 Subject Inclusion Criteria

- HIV-1 infection
- Age ≥18 years
- Ability and willingness to give informed consent.
- Plasma/Serum cryptococcal antigen (CRAG)+

3.2 Subject Exclusion Criteria

- Cannot or unlikely to attend regular clinic visits
- History of cryptococcal infection
- Symptomatic meningitis (confirmed by CSF CRAG+)
- >14 days of fluconazole therapy
- Pregnancy (confirmed by urinary or serum pregnancy test)
- Current breastfeeding
- Known allergy to amphotericin

Rationale for Criteria:

The inclusion and exclusion criteria are meant to be broad to enroll a representative sample of persons with cryptococcal infection in resource-limited areas, and thereby be broadly generalizable. Both those ART naïve, and on ART will be eligible for participation.

Amphotericin is not teratogenic. Amphotericin B is U.S. Food and Drug Administration Category B = “Animal reproduction studies have failed to demonstrate a risk to the fetus and there are no adequate and well-controlled studies in pregnant women.” The reason for exclusion, however, is that pregnant and breastfeeding women will have contraindications to standard of care fluconazole (which is teratogenic).

Selection of the Study Population

Potential research participants will be identified in outpatient HIV clinics with study team notification of cryptococcal infection patients, based on laboratory-based CrAg reflex screening from CD4 or viral load specimens. Study investigators will then contact potential subjects either in person or by mobile phone. Clinics of interest will be informed of this study and encourage to refer CRAG positive persons for consideration.

Potential participants will be evaluated in person for signs or symptoms of meningitis. If they are symptomatic, they will be offered a lumbar puncture for diagnosis of meningitis (per standard of care). If the lumbar puncture is positive (CSF CRAG+ or culture positive), they are not eligible for this study. If CSF is CRAG negative and culture negative, they may be eligible to participate.

Lumbar puncture is not a requirement for study participation in asymptomatic CRAG+ persons.

Renal failure is not an exclusion criterion for this study, as dosing of liposomal amphotericin is not adjusted for renal function.

Timing of Study Entry

The majority of potential research participants will most likely be identified via the initial plasma CRAG result. Ideally, they will be identified within 1 day of diagnosis to be offered study enrollment. Written informed consent will be obtained from participants prior to enrollment in this study.

3.2.1 Reasons for Withdrawal

Subjects are free to withdraw from participating in the study at any time upon request.

A study subject may discontinue study medicine and remain in the study due to any clinical adverse event (AE), laboratory abnormality, inter-current illness, or other medical condition or situation occurs such that continued receipt of study medicine would not be in the best interest of the subject.

4 STUDY INTERVENTION

4.1 Study Product Description: Liposomal amphotericin

Liposomal amphotericin (Brand name AmBisome®) is considered standard of care for cryptococcal meningitis treatment. All medications received in this study are considered standard of care. Liposomal amphotericin will be provided by the study. No other medications will be provided by the study.

Mechanism of Action: Binds to ergosterol altering cell membrane permeability in susceptible fungi and causing leakage of cell components with subsequent cell death. Proposed mechanism suggests that amphotericin causes an oxidation-dependent stimulation of macrophages.[56]

Pharmacodynamics/Kinetics: Exhibits nonlinear kinetics (greater than proportional increase in serum concentration with an increase in dose)

Half-life elimination: 7 to 10 hours (following a single 24-hour dosing interval); Terminal half to life: 100 to 153 hours (following multiple dosing up to 49 days)

4.1.1 Acquisition

Gilead will provide the liposomal amphotericin medication.

4.1.2 Product Storage and Stability

Storage: Unopened vials are to be stored at temperatures up to 25ºC (77ºF).

Storage of reconstitute product concentrate: The reconstituted product concentrate may be stored up to 24 hours at 2 to 8ºC (36 to 46ºF) following reconstitution with sterile water for injection. Do not freeze.

Injection of AmBisome should commence within 6 hours of dilution with 5% dextrose injection.

4.2 Dosage, Preparation and Administration of Study Investigational Product

Usual dose range 3 to 6mg/kg/day IV. Usual dosing for cryptococcal meningitis in HIV-infected patients: 6mg/kg/day.

Dose range for this study: 10mg/kg IV x1 dose.

No dosage adjustment is needed for patients with preexisting renal or hepatic impairment.

Study administration. AmBisome comes in a 50mg (1ea) vial. It is combined with cholesterol, distearoyl phosphatidylglycerol, hydrogenated soy phosphatidylcholine, sodium succinate hexahydrate, sucrose, tocopherol, and dl-alpha.

Administer via intravenous infusion over a period of ~1-2 hours.

4.2.1 Adverse reactions per FDA package insert:

In clinical trials where persons received multiple weeks of therapies, AEs included:

>10% Adverse Event Incidence:

Cardiovascular: Hypertension (8 to 20%), tachycardia (9 to 19%), peripheral edema (15%), edema (12 to 14%), hypotension (7 to 14%), chest pain (8 to 12%), localized phlebitis (9 to 11%)

Central nervous system: Chills (29 to 48%), insomnia (17 to 22%), headache (9to 20%), pain (14%), anxiety (7 to 14%), confusion (9 to 13%)

Dermatologic: Rash (5 to 25%), pruritus (11%)

Endocrine & metabolic: Hypokalemia (31 to 51%), hypomagnesemia (15 to 50%), hyperglycemia (8 to 23%), hypocalcemia (5 to 18%), hyponatremia (9 to 12%), hypervolemia (8 to 12%)

GI: Nausea (16 to 40%), vomiting (11 to 32%), diarrhea (11 to 30%), abdominal pain (7 to 20%), constipation (15%), anorexia (10 to 14%)

GU: Nephrotoxicity (14 to 47%), hematuria (14%). The incidence of any Grade $\geq$3 kidney-related AE with single dose amphotericin was 6% (1/18) in phase II trial of cryptococcal meningitis.[2] In a trial of visceral leishmaniasis, zero of 304 participants had residual nephrotoxicity at 30 days.[1]

Hematologic: Anemia (27 to 48%), leukopenia (15 to 17%), thrombocytopenia (6 to 13%)

Hepatic: Increased serum alkaline phosphatase (7% to 22%), hyperbilirubinemia (≤18%), increased serum ALT (15%), increased serum AST (13%), abnormal hepatic function tests (not specified) (4% to 13%)

Hypersensitivity: Transfusion reaction (9% to 18%)

Infection: Sepsis (7% to 14%), infection (11% to 13%)

Neuromuscular & skeletal: Weakness (6% to 13%), back pain (12%)

Renal: Increased serum creatinine (18% to 40%), increased blood urea nitrogen (7% to 21%)

Respiratory: Dyspnea (18% to 23%), pulmonary disease (14% to 18%), cough (2% to 18%), epistaxis (9% to 15%), pleural effusion (13%), rhinitis (11%)

Miscellaneous: Infusion related reactions overall: 4% to 21% including symptoms of: fever (7% to 24%), chills (6% to 24%), vomiting (4% to 16%), nausea (8% to 14%), dyspnea (5% to 10%), tachycardia (2% to 10%), hypertension (2% to 9%), vasodilation (5%), hypotension (4%), hyperventilation (1%), hypoxia (≤1%).

4.2.2 Adverse Reactions with Single Dose

The majority of adverse events related to amphotericin are due to the cumulative toxicity. In contrast, single high doses have been used for visceral leishmaniasis without toxicity. [1]

In one randomized trial of 412 patients receiving either liposomal amphotericin B 10 mg/kg given once versus amphotericin B deoxycholate given 1 mg/kg every other day for 15 doses (over 29 days).[1] Adverse events in the liposomal-therapy group were infusion-related fever or rigors (in 40%) and increased anemia or thrombocytopenia (in 2%) (see table below). In contrast, the conventional-therapy group (that received amphotericin B) developed fever or rigors (in 64%), increased anemia (in 19%), and hypokalemia (in 2%). Nephrotoxicity or hepatotoxicity developed in no more than 1% of patients in each group.

Event	Liposomal Amphotericin B (N=304)		Amphotericin B Deoxycholate (N=108)		P Value†
	Day 1–2	Day 30	During Treatment	Day 30‡	
	no. of patients (%)				
Infusion-related fever or rigors	121 (40)	0	69 (64)	0	<0.001
Increased anemia	6 (2)	4 (1)	21 (19)	18 (17)	<0.001
Increased thrombocytopenia	5 (2)	0	2 (2)	0	NS
Nephrotoxicity	2 (1)	0	1 (1)	0	NS
Hypokalemia	0	0	2 (2)	0	0.02
Hepatotoxicity	2 (1)	1 (<1)	0	1 (1)	NS

4.2.3 Warnings/Precautions

Concerns related to adverse effects:

• Anaphylaxis: Has been reported with amphotericin B-containing drugs. If a severe anaphylactic reaction occurs, the infusion should be immediately discontinued; the patient should not receive further infusions. Administer under close clinical observation during initial dosing.

• Infusion reactions: Acute reactions (including fever and chills) may occur 1 to 3 hours after starting infusions; reactions are more common with the first few doses and generally diminish with subsequent doses. Immediately discontinue infusion if a severe anaphylactic reaction occurs; the patient should not receive further infusions. Acetaminophen and/or an antihistamine can be given prior to the infusion to mitigate any reactions.

4.2.4 Pregnancy Implications

Pregnancy Risk Factor B

Adverse events were not observed in animal reproduction studies. Amphotericin crosses the placenta and enters the fetal circulation. Amphotericin B is recommended for the treatment of serious systemic fungal diseases in pregnant women; refer to current guidelines.[33]

4.2.5 Breast-Feeding Considerations

It is not known if amphotericin is excreted into breast milk. Due to its poor oral absorption, systemic exposure to the nursing infant is expected to be decreased; however, because of the potential for toxicity, breast-feeding is not recommended by the manufacturer.[57]

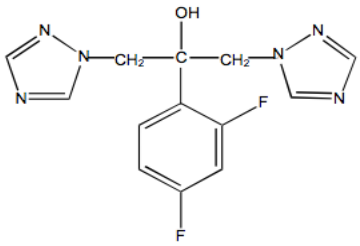
4.3 Modification of Study Intervention/Investigational Product for a Participant

Infusion reactions: Acute reactions (including fever, chills, and rigors) may occur 1 to 3 hours after starting infusions; reactions are more common with the first few doses and generally diminish with subsequent doses. Immediately discontinue infusion if a severe anaphylactic reaction occurs; the patient should not receive further infusions.

Given that only one dose is administered for this study, the patient will be monitored for the first dose for any infusion-related reaction. No additional doses are administered after this first/only dose.

4.4 Concomitant Medications: Fluconazole

Fluconazole is a synthetic triazole antifungal agent that is available in 200 mg oral tablets. Fluconazole is a white crystalline solid, which is slightly soluble in water and saline. Diflucan® tablets contain 200 mg of fluconazole and the following inactive ingredients: microcrystalline cellulose, dibasic calcium phosphate anhydrous, povidone, croscarmellose sodium, FD&C Red No. 40 aluminum lake dye, and magnesium stearate.



Typical dosing is 200-1200 mg daily. Duration of dosage depends on severity of infection. The oral bioavailability of fluconazole is ≥90%.

Fluconazole is a highly selective inhibitor of fungal cytochrome P450 dependent enzymes. It is a potent CYP2C9 inhibitor and moderate CYP3A4 inhibitor. Thus, patients who are on fluconazole and other drugs metabolized through CYP2C9 and CYP3A4 should be monitored.

Acquisition: Fluconazole may be obtained from the Ministry of Health, Diflucan Partnership Program, or any nationally registered fluconazole product. The study may provide fluconazole if there are clinic stock outs.

Product Storage and Stability: Fluconazole needs to be stored below 86°F (30°C).

Study administration: Fluconazole is an oral medication, used for the treatment of many fungal infections including cryptococcal meningitis. In this study, we will be using doses of 800 mg/day for preemptive treatment of asymptomatic cryptococcal antigenemia for 14 days. Thereafter, dosing will be reduced to 400mg/day for 8 weeks. This is standard of care per WHO and Ugandan National guidelines.

Possible side effects of the medication are rare, but include headache, rash, nausea, vomiting, diarrhea, and abdominal pain. Patients will be warned of potential side effects at study entry, and closely monitored for adverse events. Fluconazole doses of 800 mg will be divided into at least twice daily to avoid nausea and vomiting.

4.5 Accountability Procedures for the Study Intervention

Clinical personnel used in the dispensation and administration of study drugs involved in the study will adhere to GCP practices. Pharmacies utilized in the study will maintain the supply and record keeping of all study drug and antifungal dispensing.

4.6 Assessment of Subject Adherence to Study Interventions

Self-reported compliance and pharmacy records will be used to assess study drug and fluconazole adherence during outpatient management, and tablets may be counted at each clinic visit to verify self-reports of study drug adherence.

5 STUDY SCHEDULE

5.1 Screening

Potential participants will be identified through lab-based reflex CrAg screening based on CD4 counts in outpatient HIV clinics. If HIV status is unknown, a rapid HIV test will be performed. If the potential participant is a female of reproductive age, whose last menstrual period is >4 weeks prior, a urine or serum pregnancy test will be performed.

5.2 Informed Consent

Please see **Appendix A** for informed consent documents. Informed consent will be obtained by a study investigator who will provide potential subjects with an approved consent form in English/Luganda (the local language). The goal time of approaching study subjects for participation is within 5 days of diagnosing cryptococcal infection. At time of informed consent, a complete history and physical examination will be performed to verify inclusion/exclusion criteria.

The investigator will describe the purpose, risks, and benefits related to the study. Each aspect of the informed consent form will be explained in detail with the potential subject, and the potential subject will have opportunity to ask any questions regarding the study. The investigator obtaining informed consent will ask questions to assess the subject's understanding. The investigator will state that participation is voluntary and that subjects may refuse participation or withdraw at any time without prejudice to their clinical care. Persons who decline participation will be recommended to standard of care treatment for asymptomatic cryptococcal antigenemia.

If, in the opinion of the investigator, potential participants do not have appropriate comprehension, the investigator must re-explain the study. If the potential subject is deemed in the opinion of the investigator to be unable to give informed consent, they will not be enrolled in the study. Altered mental status may be a sign of cryptococcal meningitis. In order to enroll persons with asymptomatic cryptococcal infection, before onset of meningitis, only those with capacity to consent will be enrolled.

5.3 Enrollment

On study enrollment, patients will be assessed to confirm that they meet eligibility criteria. They will have a full physical examination. If patient meets all inclusion criteria, and no exclusion criteria are met, they will undergo the informed consent process.

Thereafter they will be randomized to receive

a) Liposomal amphotericin 10mg/kg x1 (single dose) + fluconazole 800mg daily for 14 days OR

b) Fluconazole 800mg daily for 14 days (standard of care).

Baseline labs (CBC, Creatinine, Na, K) will be obtained, so that AEs can be assessed at future visits. Baseline assessment will include drug history, ART history, baseline symptoms, and physical examination. If CD4 test has not been documented in the prior 4 weeks, this may be repeated.

Regardless of whether fluconazole was prescribed per routine care prior to enrollment, fluconazole 800mg daily will be initiated on day 1 (enrollment) and continued for 14 days thereafter.

5.4 Follow-up

Patients will return for follow up on day 7 after enrollment for follow up labs, and to evaluate for signs, symptoms or meningitis.

At day 14 they will return to initiate ART and transition fluconazole to 400mg daily. ART drug choice will be per clinical standard of care in the clinic.

Follow up visits will occur at weeks 1, 2, and 4 to assess for interim AEs, incidence of meningitis. Thereafter, participants will be followed at weeks 8, and 12 to assess vital status and medication adherence. If they do not return to clinic, they will be contacted by phone to confirm if they are (a) alive and in care and (b) incidence of meningitis. Additionally, their medical chart will be accessed to clarify this information. Intermittent phone contact will occur between 16 and 24 weeks to ensure patients are alive and in care.

At these research visits, participants' transportation expenses will be reimbursed.

5.5 Final Study Visit

At study termination, we will document vital status, and/or reason for study termination Reasons for study termination are 6-month study completion, withdrawal of consent, death, or lost to follow up. Analysis is by intention to treat. At the study termination visit, the following will be documented:

- Interval history
- Review of medications and adherence
- Vital signs
- AEs
- Complete physical exam

6 STUDY PROCEDURES/EVALUATIONS

6.1 Clinical Evaluations

Screening

Plasma CRAG screening will be performed using CRAG Lateral flow assay (Immy, Norman, Oklahoma USA) to identify those with asymptomatic cryptococcal infection. This may be done reflexively in the lab, on remaining CD4 specimen, plasma sample after VL testing, or may be done as a point-of-care test in the clinic. The patient will be assessed to exclude signs or symptoms of meningitis. If there is clinician concern for meningitis, a lumbar puncture should be performed.

Documented cryptococcal infection will be defined as blood CRAG positive.

At screening, a history of the present illness, medical history, ART history, physical and neurologic examination will be performed and documented on the 'Screening CRF.'

If a potential participant is found to be blood CRAG+, study staff will verbally screen for inclusion/exclusion criteria prior to the informed consent process.

- HIV-status must be documented with a screening rapid test, if not already known.
- For women of childbearing age who could potentially be pregnant (last menstrual period >4 weeks), a urine (or serum) pregnancy test is required

Enrollment

If the patient meets the eligibility criteria, they will be approached by study staff for informed consent. Those who are willing will complete the informed consent process and sign the informed consent documents.

Summary of events during enrollment visit:

- Confirmation of study eligibility
- Informed consent
- Baseline labs
- Randomization (potential study intervention)

At enrollment, current signs/symptoms occurring must be recorded. Baseline labs will be obtained. All grade 3 or 4 adverse events must be recorded on the enrollment CRF, so that **new** events may be correctly assessed and documented.. Grade 3 or 4 adverse events are commonly expected among participants at study entry and will likely include: fatigue, weight loss, anorexia, ± nausea.

Those enrolled will be randomized to receive liposomal amphotericin (10mg/kg x1) on the day of enrollment, in addition to standard of care fluconazole or standard of care fluconazole alone.

Participants may be given paracetamol and/or an antihistamine before or during the infusion to mitigate any infusion reactions. All patients will receive 1L of normal saline over 1 to 2 hours prior to the AmBisome infusion to minimize the risk of nephrotoxicity, along with electrolyte supplementation, per WHO guidelines.

For those randomized to receive AmBisome, assessment for infusion reactions will occur after the infusion.

Follow up Visits

Day 7
A study medical officer will assess:

- Interval history
- Vital Signs
- Focused physical examination, directed by symptoms
- Medications and adherence (as appropriate)
- Adverse events (AEs)

During stage 1, laboratory monitoring at day 7 will include:

- CBC
- Serum creatinine
- Serum chemistry (Na, K)

During stage 1, laboratory monitoring will only occur at baseline (screening) and on day 7. If there are significant abnormalities in the above labs on day 7, that were not already present at enrollment, further monitoring may occur to ensure resolution, and determine association with the study drug.

Day 14
A study medical officer will assess:

- Interval history
- Vital Signs
- Focused physical examination, directed by symptoms
- Medications and adherence (as appropriate)
- Adverse events (AEs)

During stage 1, laboratory monitoring at day 14 will include:

- CBC
- Serum creatinine
- Serum chemistry (Na, K)

ART counseling and initiation will occur at this visit.
From this point forward, all care = standard of care *per Ugandan and international guidelines.*

Week 4
A study medical officer will assess:

- Interval history (meningitis event)
- Vital Status
- Focused physical examination, directed by symptoms
- Medications and adherence (as appropriate)

- Adverse events (AEs)

Weeks 8 and 12
A study medical officer will assess:

- Interval history (meningitis event)
- Vital Status
- Focused physical examination, directed by symptoms
- Medications and adherence (as appropriate)
- Adverse events (AEs)

Phone Contact

Phone contact will occur with any missed visits. Additionally, we will contact patients at approximately weeks 16 and, 20 to assess vital status, and for any meningitis events.

Week 24
A study medical officer will assess:

- Interval history (meningitis event)
- Vital Status

This may occur via phone contact.

Sick Visit or Premature Discontinuation
A study medical officer will assess:

- Interval history
- Vital Signs
- Focused physical examination, directed by symptoms
- Adverse events (AEs)

If there is concern for meningitis at any point, a lumbar puncture may be pursued if thought to be clinically appropriate. Remaining CSF specimen may be stored for future testing.

6.2 Schedule of events

Procedures		Enrollment	Wk 1	Wk 2	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 24	Sick visit/ Premature discontinuation
Signed Consent Form		X									
Assessment of Eligibility Criteria		X									
Randomization to intervention: AmBisome		X									
Fluconazole mg		800	800	400	400	400	200			Stop	
Initiate ART				Target							
Study Visit		X	X	X	X	X	X				
Telephone Contact								X#	X#	X#	
Physical Examination		X									
Symptom-Directed Exam			±	±	±	±	±			±	±
Assessment of Adverse Events		X*	X	X	X	X	X	X	X	X	X
Clinical Laboratory	Na, K, Creatinine	X	Stage 1	Stage 1							
	ALT, Tbili	X	Stage 1	Stage 1							
	CBC	X	Stage 1	Stage 1							
	CD4 test	±									
	Pregnancy test	±									
	CSF analysis										±
Specimen Storage		X	Stage 1	Stage 1							±
Assess Vital Status and meningitis events			X	X	X	X	X	X	X	X	X

* Post-infusion evaluation for adverse events.

Stage 1 labs represent additional safety monitoring that may be eliminated in stage 2 of this study, if incidence of new lab AEs is ≤6% and/or in agreement with the Data Monitoring Committee after the first 100 persons of Stage 1 are enrolled.

These visits are planned by phone. But in-person visits are allowable.

Pregnancy testing will occur at screening if pregnancy is suspected (last menstrual period >4 weeks) at baseline or at any follow up visit. CD4 testing will occur at enrollment if CD4 testing has not otherwise been performed/documented in the prior 4 weeks to enrollment. ART will be initiated per clinic standard of care (goal is at 2 weeks). Week 24 visits may occur by phone or from chart review.

6.3 Adverse Events

Adverse Event: ICH E6 defines an adverse event (AE) as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

Given that the intervention occurs once only, at enrollment, AEs will be monitored at enrollment, weeks 1, weeks 2 post enrollment, and additionally for any sick visits. Thereafter AEs would be unlikely to be related to the intervention. Beyond the one time infusion, all care would be per routine HIV care per Ugandan (and WHO) HIV guidelines. AEs that occur after 2 weeks are more likely due to underlying HIV disease, and unlikely related to the experimental intervention.

For purposes of regulatory reporting, all SAEs occurring within two weeks of enrollment are reportable to regulatory authorities, per Ugandan regulations. Thereafter all follow up is observational.

All AEs within the first 2 weeks of enrollment including local and systemic reactions not meeting the criteria for "serious adverse events" should be captured on the appropriate CRF (including any sponsor SAE). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring within 2 weeks of the intervention must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution or stabilization.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates or recurs at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product. Per NIH NIAID DAIDS policy, all symptoms must be assessed as to whether they are clinically significant and reportable (i.e. Severity Grade $\geq$3). *For this study, we will collect AEs that occur in both arms for the duration of the study, but will only report AEs to regulatory agencies that occur within 2 weeks (14 days) of enrollment or at an unscheduled sick visit. Events that occur after this 2 week period are unlikely to be related to the intervention.* Events that occur after this two week period will be collected and reported in aggregate annually.

Stage 1: Stage 1 labs represent additional safety monitoring that may be eliminated in stage 2 of this study if incidence of new lab AEs is $\leq$6% and/or in agreement with the Data Monitoring Committee after the first 100 persons of Stage 1 are enrolled.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: associated or not associated. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Associated – The event is temporally related to the administration of the study product and no other etiology explains the event.

- Not Associated – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

Severity of Adverse Events

The term severity is defined as the intensity grade or level for a specific event, i.e., mild, moderate, severe, or life threatening. Importantly, severity is *not* the same as seriousness, which is based on participant/event *outcome or action* criteria usually associated with events that pose a threat to a subject's life or functioning (ICH E2A). The *Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (v2.0, Nov 2014)* will be used.

Three severity grades for AEs are to be recorded:

Grade 3 = Severe
Grade 4 = Potentially life-threatening
Grade 5 = Death

Expected Adverse Events

Expected events include:

- Early death due to cryptococcosis (up to 70% of enrolled subjects)
- Symptoms due to meningitis, IRIS, or relapse
- Cryptococcal IRIS events (~40%)
- Hospitalization or death due to IRIS or cryptococcal meningitis (20-25%)
- Common AIDS-related opportunistic infections and complications, including tuberculosis
- Anemia
- Infusion reactions related to the study drug (fever, chills, rigors)

These expected events will be reported to the local IRB, study sponsor, and National Drug authority per their regulations.

Adverse events will be collected from study entry through 24 weeks post-intervention. Once an AE occurs, the participant must be followed until AE resolution or stabilization. While adverse events will be collected for the duration of the study, adverse events will be reported to regulatory agencies only within two weeks of enrollment or at a sick visit.

6.3.1 Serious Adverse Events

AEs are classified as serious or non-serious. A S*erious adverse event* is:

- fatal
- life-threatening
- requires re-hospitalization (after hospital discharge for the initial, pre-existing cryptococcal meningitis)
- requires prolongation of the initial hospitalization by >3 days
- results in persistent or significant disability or incapacity
- results in congenital anomaly or birth defect
- an important medical event that may jeopardize the subject or may require immediate intervention to prevent one of the other outcomes listed in the definition above

All AEs that do not meet any of the criteria for serious will be classified as *non-serious adverse events*. A standard outpatient medical problem, e.g. malaria, which in theory if left untreated could result in a fatal or life-threatening event, is not by itself a SAE. If the event is of sufficient severity to result in hospitalization, then the AE is a SAE.

All SAEs identified within 24 weeks of enrollment will be:

- recorded on the appropriate AE CRF (as provided by study sponsor)
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

6.3.2 Reporting Procedures

Given that the intervention occurs once only, at enrollment, AEs will be monitored at enrollment, weeks 1, and weeks 2, and at any non-scheduled sick visits post enrollment. Thereafter AEs would be unlikely to be related to the intervention. AEs will be collected for the duration of study enrollment, but will be reported to the appropriate regulatory agencies, only if they occur within 2 weeks of enrollment, or at a sick visit. Beyond the one time infusion, care would be per routine HIV care. AEs that occur after 2 weeks are more likely due to underlying HIV disease, and will not be relevant to this study. .

To comply with national regulations, reports of all ***serious unexpected*** adverse events will be submitted to the IRB with oversight within 3 working days of the study site awareness of the AE. Reporting will be via the 'Adverse Event Reporting CRF.' A copy must be

forwarded to the study sponsor. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder. At the time of the initial report, the following information will be provided:

- Study identifier
- Study Site
- Subject number
- Date of event
- A description of the event
- Medical treatments given / discontinued
- Working diagnosis
- Current vital status

Within the 7 working days following the event, the investigator will provide further information on the AE Reporting CRF in the form of a written narrative. This will be documented along with any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious AEs will be provided promptly to the study sponsor and local IRB of record.

6.3.3 Reporting of Pregnancy

Women with cryptococcal infection who are currently pregnant will be excluded from this study as standard of care fluconazole is teratogenic.

- Women of childbearing potential must use a method of contraception while in the study
- All participants must agree not to participate in a conception process (e.g., active attempt to become pregnant or to impregnate, donate sperm, in vitro fertilization).

Women of childbearing potential who after enrollment are found to not be using contraception or are breast-feeding will immediately stop fluconazole (per clinic standard of care). Pregnancy testing should be repeated as clinically indicated.

In the event that a subject becomes pregnant after enrolling in the study, she will be referred to the local antenatal clinic and closely followed up by the study team as well. The study team itself will not provide antenatal care.

Dependent on the duration of antifungal therapy, CD4 count, efficacy of ART, and consultation with cryptococcal expert consultants, a recommendation will be made for the pregnant patient on the risks/benefits of azole continuation, discontinuation, or dose reduction. The executive committee's recommendation is only meant to be a provision of advice, which physicians can share with the subjects. The decision on the management of the subject is at the discretion of the physician and ultimately the subject.

6.3.4 Study medicine discontinuation

This is not applicable to this study, as only one dose of liposomal amphotericin will be administered. If there is concern for anaphylaxis during administration, the study medicine will be discontinued immediately. Acetaminophen and antihistamines will be available to participants before and during infusion in case of reactions such as fevers, chills, discomfort, rigors. Such reactions are expected in approximately 40% of participants, and will only last for the duration of the infusion (approximately 1 hour).[1]

7 CLINICAL AND SAFETY MONITORING

7.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the sponsor, ICH E6 and, when appropriate, regulatory guidelines.

Monitoring will be conducted by the designee of the study sponsor. This may include monitors from the Infectious Diseases Institute. Monitors will assess:

- Informed consent documentation
- entry eligibility criteria documentation
- missed study visits
- completion of the correct CRFs at the specified study visit
- signing/dating of CRFs and GCP compliance
- completion of protocol specified laboratory tests

7.2 Planned Interim Safety Reviews

A Data Safety Monitoring Board (DSMB) will be in place to oversee safety. They will conduct an initial protocol review. Thereafter, the DSMB will meet after 100 participants have been enrolled with 4 weeks of follow-up to assess for safety.

There will be an enrollment pause to allow time for the 4 weeks of follow up, analysis, and then a DSMB meeting.

Thereafter, the DSMB will conduct reviews every 6-12 months of enrollment and will meet at least annually. Based on the accumulating data, the safety committee may meet more frequently.

DSMB Reports will include the information contained in the Progress Reports and Clinical Quality Management Plan reports as well as an analysis for the primary endpoint.

Reports will be prepared with pooled demographic and trial progress data, data prepared by randomization group for demographics of enrolled participants and the primary outcome. Baseline demographic features of each study arm will be summarized, with statistical testing as appropriate for nominal and continuous variables to assure adequacy of randomization.

7.3 Data Safety Monitoring Board

A Data Safety Monitoring Board will be assembled to review:

- Unexpected Adverse Events
- Potential AmBisome-related Adverse Events
- Early Mortality + SAE Independent Review

A quorum of members is required for the early safety independent review. For adjudication of unexpected adverse events or potential AmBisome-related AEs, adjudication by at least two members is necessary with consensus agreement.

The Data Safety Monitoring Board shall be empowered to:

1) pause or halt further enrollment;
2) request further interim safety reviews.

The Data Safety Monitoring Board will be independently chaired and will consist of an independent body of investigators not involved with the trial, and a biostatistician from the study team. A sponsor representative may observe as a non-voting member of the committee.

The PhD biostatistician will prepare summary progress reports on tolerability, safety, and efficacy within approximately 30 days of each 100 participants being enrolled.

Data are unblinded, thus the PI, on site investigators, and other team members will additionally monitor for any unexpected concern. If concerns are raised, this will prompt an unscheduled DSMB evaluation.

7.3.1 Unscheduled Safety Reviews

First, if there are unexpected safety concerns of serious and potentially related AEs, these AEs will be reviewed by the Data Safety Monitoring Board, and reported to the relevant regulatory authorities with oversight. Unexpected safety concerns may result in suspension of further trial interventions. The study PI, DSMB chair, and sponsors each retain the authority to suspend additional enrollment and study interventions for the entire study. Examples of findings that might trigger a safety review are the number of SAEs overall, the number of occurrences of a particular type of SAE, severe AEs/reactions, or increased frequency of events.

7.4 Stage 1 Review

The overall conduct of the trial will be reviewed with Stage 1 including the base statistical assumption of the trial of ~75% meningitis free survival; tolerability/feasibility of liposomal amphotericin infusion; adverse event rate; and potential additional efficacy. Based on the cumulative Stage 1 experience, a Go / No Go decision will be made for Stage 2. This interim Stage 1 review will be conducted at approximately ~2 months after the 100th subject is enrolled to assess for safety and efficacy in order to make a Go / No Go Decision to proceed to Stage 2. At this interim analysis, participants will be censored based on their last follow up date. We anticipate proceeding with Stage 2 as soon as possible after the DSMB recommendations, if this is what is suggested.

7.5 Halting guidelines:

A modified Haybittle–Peto boundary will be used for early stoppage in stage 2 (phase 3 of the study) due to either excess harm or improved efficacy for the meningitis-free survival primary endpoint. The threshold for stopping will be P=0.01 at interim analyses for early stoppage; and a p=0.05 at the final analysis. This modification is due to the strong biological premise that additional antifungal therapy for a person with a disseminated fungal infection, when P<0.01, this is unlikely to be due to random chance. Using a more stringent stopping boundary (e.g. P<0.001) would expose more research participants to unnecessary harm, which runs counter to the research ethics principle of beneficence.

If the trial is halted early for efficacy, future eligible participants will be given the preferred intervention as an observational cohort, until the final randomized participant exits the study. As such this will provide an orderly end to the trial, as opposed to stopping the trial for efficacy and then providing no further care to the population from which the trial's volunteers come.

8 STATISTICAL CONSIDERATIONS

8.1 Study Hypotheses

We hypothesize that addition of single dose liposomal amphotericin to standard fluconazole for asymptomatic cryptococcal antigenemia will improve 6-month meningitis-free survival. We hypothesize that single dose liposomal amphotericin will be well tolerated and will have an excellent safety profile.

8.2 Randomization

We will use a permutated block randomization in a 1:1 allocation (n=300 per arm). Randomization will be stratified by site. Randomization codes will be developed and held by the trial biostatistician at the University of Minnesota.

Randomization schedules will be supplied to the pharmacy at each clinical trial site. Detailed instructions for study drug processing, labeling and dispensing are included in the Pharmacy SOP. This study is not blinded.

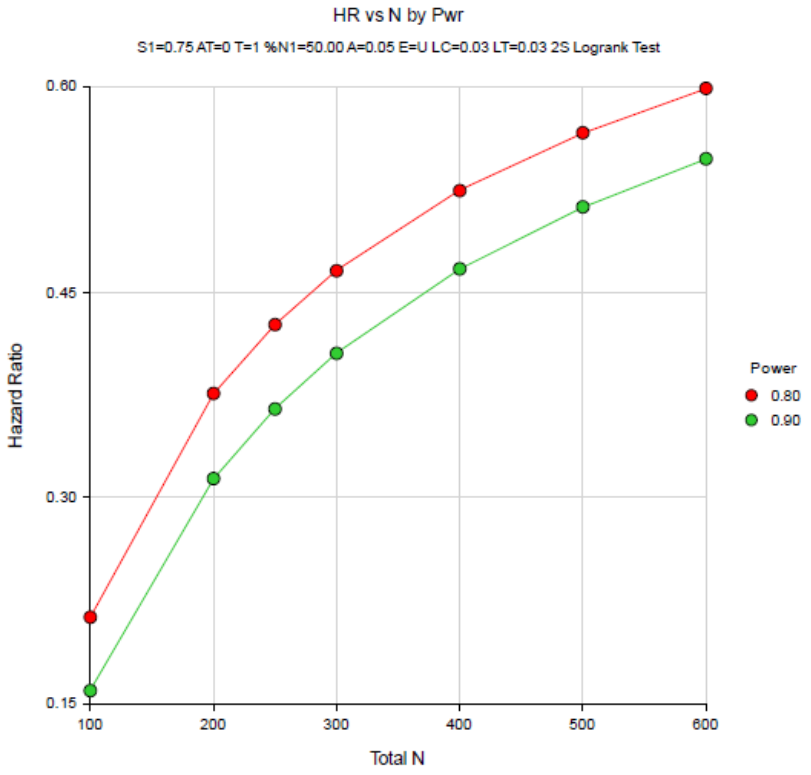
8.3 Sample Size Considerations

From our prior CRAG screening trial we estimate that 6-month meningitis-free survival (i.e. no breakthrough meningitis and alive) among those asymptomatic CRAG+ receiving standard of care will be 75%. CrAg negative 6-month survival among persons with a CD4<100 cells/μL and ART is 89% in Uganda.

With 300 subjects receiving liposomal amphotericin + standard of care and 300 receiving standard of care alone, a two-sided log-rank test achieves 80% power at 0.05 significance to detect a hazard ratio of 0.60, which translates to an approx. survival of 84% in the intervention group (~9% absolute difference). Enrolling 600 CRAG+ persons over 4 years requires us to enroll ~14 people per month. This is the largest probable sample size.

However, as the effect size is larger (i.e. better survival), the same size decreases necessary to prove superiority. At present the likely effect is unknown. A 15% mortality benefit would require a n=200 subject in total trial. After completion of stage 1 (n=100), a sample size re-estimation will occur. Based on multiple considerations including feasibility, safety, and possible efficacy, a decision to continue into stage 2 will then occur.

Power	Control group n	Intervention group n	Total n	Hazard Ratio (HR)	Control proportion survives	Intervention proportion survives
0.8	50	50	100	0.213	0.75	0.94
0.8	100	100	200	0.376	0.75	0.90
0.8	125	125	250	0.426	0.75	0.89
0.8	150	150	300	0.466	0.75	0.87
0.8	200	200	400	0.524	0.75	0.86
0.8	250	250	500	0.566	0.75	0.85
0.8	300	300	600	0.599	0.75	0.84



To enroll 600 people in total, with a CrAg prevalence rate of approximately 8%, we anticipate screening 7500 persons with a CD4<100 cells/μL.

8.4 Final Analysis Plan

We will evaluate 6-month cryptococcal meningitis-free survival in the asymptomatic CRAG+ participants who receive liposomal amphotericin + standard of care fluconazole, compared to those who receive fluconazole alone.

We will compare survival in a time-to-event analysis for cryptococcal-free survival using Log-rank test with survival displayed as a Kaplan-Meier curve. Analysis will be by intention-to-treat. Persons who develop meningitis are failures. Persons lost to follow up in the first 10 weeks will be considered failures. Persons lost to follow up after the 10 week study visit, will be actively traced. If unfound, they will be censored.

All asymptomatic CrAg+ persons enrolled will be included in the denominator. Persons symptomatic at baseline would not be enrolled but referred to the hospital for meningitis diagnosis and treatment.

One subgroup of interest are the asymptomatic CrAg+ persons with high titers ($\geq$1:160). From prior cohorts, we expect 40% of asymptomatic CrAg+ persons to have high titers (n=120 in each group). High titer persons have 37% 6-month mortality with standard of care fluconazole compared to asymptomatic CrAg+ persons with low titers who have 17% 6-month mortality. We will compare 6-month mortality between those in the intervention arm with high titers, and those in the control arm with high titers. While we are not powered to show a statistically significant difference in this subgroup, this exploratory analysis will be important for future studies, and cost-effectiveness analyses.

Any parameters found to be potentially different between randomization groups (P<0.1) and associated with outcome may be included in a multivariable adjusted Cox regression analysis.

8.5 Analysis Plan Secondary and Other Endpoints

- **6-month Survival Time**
  Survival time will be analyzed using Log-rank test and displayed via Kaplan-Meier survival curve. Persons lost to follow up will be considered failures. All participants will be censored at 6 months.
- **Incidence of Symptomatic Cryptococcal Meningoencephalitis**

  The cumulative incidence function will compare the randomized groups for incidence of symptomatic cryptococcal-related meningitis/encephalitis, which will account for the competing risk of death.[31]

  Symptomatic meningoencephalitis will be defined as meningitis symptoms plus:

  - *Cryptococcus* culture positive meningitis,
  - CSF CrAg+, or
  - Cryptococcoma(s) by neuroimaging or post mortem exam.[58]
- **Incidence of Adverse Events**

The cumulative incidence function will compare the randomized groups for incidence of Grade 3, 4, 5, and Serious AEs, which will account for the competing risk of death.[31]

Cryptococcal-related deaths and incident cryptococcal meningitis will not be included in the analysis of AEs, but these will be analyzed as separate endpoints (as above).

- **Incidence of Laboratory Adverse Events**

  The cumulative incidence function will compare the randomized groups for incidence of laboratory adverse events of Grade 3, 4, 5, which will account for the competing risk of death.[31] A laboratory value which results in clinical action meeting the definition of a Serious AEs (e.g. hospitalization), is a Serious AE and will be also included above.

  Asymptomatic / minimally symptomatic lab abnormalities which do not result in hospitalization will be summarized herein. Based on the lab monitoring, this includes hematologic, electrolyte, renal, and liver lab testing labnormalities.

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 Ethical Standard

This study is to be conducted according to US and international standards of Good Clinical Practice (International Conference on Harmonization guidelines), Declaration of Helsinki, and International Ethical Guidelines for Biomedical Research Involving Human Subjects, applicable government regulations for Uganda, US, and Institutional research policies and procedures. All investigators must have received human subject protection and GCP training prior to human subject involvement.

9.2 Institutional Review Board

Prior to the initiation of the study at each clinical research site, the protocol, all informed consent forms and the participant Information materials will be submitted to and approved by the IRB of record. Likewise, any future amendments to the study protocol will be approved by the study sponsor and then submitted and approved by each site's IRB before implementation. Please refer to Appendix A for informed consent documents.

This protocol and any amendments will undergo review and approval by the Human Subjects Board at the University of Minnesota under U.S. Federal Wide Assurance FWA00000312 and with the local IRB in Uganda.

9.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continuing throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product. Consent forms will be IRB-approved and the subject will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. The subjects will sign the informed consent document prior to any procedures being done specifically for the study. The subjects should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

9.4 Subject Confidentiality

Documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party,

without prior written approval of the participant except as necessary for monitoring by the IRB and the study sponsor.

9.5 Study Discontinuation

In the event that the study is discontinued, subjects will receive standard-of-care fluconazole for cryptococcal infection.

10 DATA HANDLING AND RECORD KEEPING

10.1 Confidentiality

Information about study subjects will be kept confidential. For research uses, all data will be de-identified and coded with a study number.

10.2 Source Documents

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents Study data forms will be digitally scanned for permanent record keeping and to enable rapid resolution of any discrepancies.

10.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. All entries will be printed legibly in black ink. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it. These case record forms will be entered via DataFax System. Study records will be digitally scanned for permanent recording, and review by oversight bodies.

Once subjects enter the study, all follow up encounters will be documented on structured source data fax CRFs documents to be used for the trial.

10.4 Data Entry

Data entry will occur via the Data Fax system. Study specific forms will be harmonized between all study sites, with DataFax system enabling multi-site data management. The overall coordination will be the responsibility of the principal investigator.

10.5 Records Retention

The investigator will retain study essential source documents for at least 5 years after the completion of the study. Digital images of the source documents will be retained for an indefinite period.

10.6 Quality Control and Quality Assurance

The DataFax system incorporates quality assurance and quality control system for complete record keeping.

10.7 Data Management Responsibilities

The UMN data manager has direct responsibilities for data management of the DataFax database with oversight by the study biostatistician and ultimately principal investigator.

10.8 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, Good Clinical Practice (GCP), or Manual of Procedures requirements. The noncompliance may be either on the part of the subject or the investigator/staff. Because of deviations, corrective actions are to be developed by the site and implemented promptly.

Dose reduction due tolerability problems is not a protocol deviation (but is the primary endpoint). Prescribing the wrong dose is a protocol deviation.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be promptly reported via CRFs, including missed study visits.

11 LITERATURE REFERENCES

1. Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW. Single-dose liposomal amphotericin B for visceral leishmaniasis in India. *N Engl J Med* 2010; **362**(6): 504-12.
2. Jarvis JN, Leeme TB, Molefi M, et al. Short Course High-dose Liposomal Amphotericin B for HIV-associated Cryptococcal Meningitis: A phase-II Randomized Controlled Trial. *Clin Infect Dis* 2018.
3. Park BJ, Wannwmuehler KA, Marston BJ, Govender N, Pappas PG, Chiller TM. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009; **23**(4): 525-30.
4. French N, Gray K, Watera C, et al. Cryptococcal infection in a cohort of HIV-1-infected Ugandan adults. *AIDS* 2002; **16**(7): 1031-8.
5. Okongo M, Morgan D, Mayanja B, Ross A, Whitworth J. Causes of death in a rural, population-based human immunodeficiency virus type 1 (HIV-1) natural history cohort in Uganda. *Int J Epidemiol* 1998; **27**(4): 698-702.
6. Corbett EL, Churchyard GJ, Charalambos S, et al. Morbidity and mortality in South African gold miners: impact of untreated disease due to human immunodeficiency virus. *Clin Infect Dis* 2002; **34**(9): 1251-8.
7. Holmes CB, Losina E, Walensky RP, Yazdanpanah Y, Freedberg KA. Review of human immunodeficiency virus type 1-related opportunistic infections in sub-Saharan Africa. *Clin Infect Dis* 2003; **36**(5): 652-62.
8. Rajasingham R, Smith RM, Park BJ, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. *Lancet Infect Dis* 2017; **17**(8): 873-81.
9. Jarvis JN, Meintjes G, Williams A, Brown Y, Crede T, Harrison TS. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis* 2010; **10**: 67.
10. Mirza SA, Phelan M, Rimland D, et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000. *Clin Infect Dis* 2003; **36**(6): 789-94.
11. UNAIDS. Access to Antiretroviral Therapy in Africa: Status report on progress towards the 2015 targets. *Geneva* 2013: www.unaids.org/en/resources/documents/2013.
12. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS* 2008; **22**(15): 1897-908.
13. Siedner MJ, Ng CK, Bassett IV, Katz IT, Bangsberg DR, Tsai AC. Trends in CD4 count at presentation to care and treatment initiation in sub-Saharan Africa, 2002-2013: a meta-analysis. *Clin Infect Dis* 2015; **60**(7): 1120-7.
14. Tenforde MW, Mokomane M, Leeme T, et al. Advanced Human Immunodeficiency Virus Disease in Botswana Following Successful Antiretroviral Therapy Rollout: Incidence of and Temporal Trends in Cryptococcal Meningitis. *Clin Infect Dis* 2017; **65**(5): 779-86.
15. Castelnuovo B, Manabe YC, Kiragga A, Kamya M, Easterbrook P, Kambugu A. Cause-specific mortality and the contribution of immune reconstitution inflammatory

syndrome in the first 3 years after antiretroviral therapy initiation in an urban African cohort. *Clin Infect Dis* 2009; **49**(6): 965-72.
16. Lawn SD, Bekker LG, Myer L, Orrell C, Wood R. Cryptococcocal immune reconstitution disease: a major cause of early mortality in a South African antiretroviral programme. *AIDS* 2005; **19**(17): 2050-2.
17. Etard JF, Ndiaye I, Thierry-Mieg M, et al. Mortality and causes of death in adults receiving highly active antiretroviral therapy in Senegal: a 7-year cohort study. *AIDS* 2006; **20**(8): 1181-9.
18. Moore D, Yiannoutos C, Musick B, et al. Determinants of mortality among HIV-infected individuals receiving home-based ART in rural Uganda. *Abstracts of the 14th Conference on Retroviruses and Opportunistic Infections, Los Angeles USA February 2007* 2007; **Abstract #34**.
19. Kambugu A, Castelnuovo B, Wandera B, Kiragga A, Kamya MR. Antiretroviral therapy in an urban African cohort does not prevent significant early mortality. *Abstracts of the 4th IAS Conference on HIV Pathogenesis, Treatment and Prevention International AIDS Society Sydney, Australia July 2007 Abstract #WEPEB055* 2007.
20. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Trop Med Int Health* 2007; **12**(8): 929-35.
21. Lawn SD, Harries AD, Wood R. Strategies to reduce early morbidity and mortality in adults receiving antiretroviral therapy in resource-limited settings. *Curr Opin HIV AIDS* 2010; **5**(1): 18-26.
22. Organization WH. Guidelines for the diagnosis, prevention, and management of cryptococcal disease in HIV-infected adults, adolescents and children. . 2018.
23. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. *Lancet* 2015; **385**(9983): 2173-82.
24. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/mcL who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010; **51**: 448–55.
25. Letang E, Muller MC, Ntamatungiro AJ, et al. Cryptococcal antigenemia in immunocompromised HIV patients in rural Tanzania: A preventable cause of early mortality. *Open Forum Infect Dis* 2015; **2**(2): ofv046.
26. Jarvis JN, Harrison TS, Lawn SD, Meintjes G, Wood R, Cleary S. Cost effectiveness of cryptococcal antigen screening as a strategy to prevent HIV-associated cryptococcal meningitis in South Africa. *PLoS One* 2013; **8**(7): e69288.
27. Butler EK, Boulware DR, Bohjanen PR, Meya DB. Long term 5-year survival of persons with cryptococcal meningitis or asymptomatic subclinical antigenemia in Uganda. *PLoS One* 2012; **7**(12): e51291.
28. Jarvis JN, Meintjes G, Wood R, Harrison TS. Testing but not treating: missed opportunities and lost lives in the South African antiretroviral therapy programme. *AIDS* 2010; **24**(8): 1233-5.

29. Lawn SD, Myer L, Harling G, Orrell C, Bekker LG, Wood R. Determinants of mortality and nondeath losses from an antiretroviral treatment service in South Africa: implications for program evaluation. *Clin Infect Dis* 2006; **43**(6): 770-6.
30. Ingle SM, May M, Uebel K, et al. Outcomes in patients waiting for antiretroviral treatment in the Free State Province, South Africa: prospective linkage study. *AIDS* 2010; **24**(17): 2717-25.
31. Mfinanga S, Chanda D, Kivuyo SL, et al. Cryptococcal meningitis screening and community-based early adherence support in people with advanced HIV infection starting antiretroviral therapy in Tanzania and Zambia: an open-label, randomised controlled trial. *Lancet* 2015.
32. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america. *Clin Infect Dis* 2010; **50**(3): 291-322.
33. Masur H, Brooks JT, Benson CA, Holmes KK, Pau AK, Kaplan JE. Prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: Updated Guidelines from the Centers for Disease Control and Prevention, National Institutes of Health, and HIV Medicine Association of the Infectious Diseases Society of America. *Clin Infect Dis* 2014; **58**(9): 1308-11.
34. Muzoora CK, Kabanda T, Ortu G, et al. Short course amphotericin B with high dose fluconazole for HIV-associated cryptococcal meningitis. *J Infect* 2012; **64**(1): 76-81.
35. Livermore J, Howard SJ, Sharp AD, et al. Efficacy of an abbreviated induction regimen of amphotericin B deoxycholate for cryptococcal meningoencephalitis: 3 days of therapy is equivalent to 14 days. *MBio* 2014; **5**(1): e00725-13.
36. Vogelsinger H, Weiler S, Djanani A, et al. Amphotericin B tissue distribution in autopsy material after treatment with liposomal amphotericin B and amphotericin B colloidal dispersion. *J Antimicrob Chemother* 2006; **57**(6): 1153-60.
37. Molefi M, Chofle AA, Molloy SF, et al. AMBITION-cm: intermittent high dose AmBisome on a high dose fluconazole backbone for cryptococcal meningitis induction therapy in sub-Saharan Africa: study protocol for a randomized controlled trial. *Trials* 2015; **16**: 276.
38. Sinha PK, Bhattacharya S. Single-dose liposomal amphotericin B: an effective treatment for visceral leishmaniasis. *Lancet Glob Health* 2014; **2**(1): e7-8.
39. Morawski B, DR; B, al. e. Pre-ART Cryptococcal Antigen Titer Associated with Preemptive Fluconazole Failure. *CROI* 2016.
40. Gubbins PO, Amsden JR, McConnell SA, Anaissie EJ. Pharmacokinetics and buccal mucosal concentrations of a 15 milligram per kilogram of body weight total dose of liposomal amphotericin B administered as a single dose (15 mg/kg), weekly dose (7.5 mg/kg), or daily dose (1 mg/kg) in peripheral stem cell transplant patients. *Antimicrob Agents Chemother* 2009; **53**(9): 3664-74.
41. Mehta P, Vinks A, Filipovich A, et al. High-dose weekly AmBisome antifungal prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation: a pharmacokinetic study. *Biol Blood Marrow Transplant* 2006; **12**(2): 235-40.
42. Hamill RJ, Sobel JD, El-Sadr W, et al. Comparison of 2 doses of liposomal amphotericin B and conventional amphotericin B deoxycholate for treatment of AIDS-

associated acute cryptococcal meningitis: a randomized, double-blind clinical trial of efficacy and safety. *Clin Infect Dis* 2010; **51**(2): 225-32.
43. Hope WW, Goodwin J, Felton TW, Ellis M, Stevens DA. Population pharmacokinetics of conventional and intermittent dosing of liposomal amphotericin B in adults: a first critical step for rational design of innovative regimens. *Antimicrob Agents Chemother* 2012; **56**(10): 5303-8.
44. O'Connor L, Livermore J, Sharp AD, et al. Pharmacodynamics of liposomal amphotericin B and flucytosine for cryptococcal meningoencephalitis: safe and effective regimens for immunocompromised patients. *J Infect Dis* 2013; **208**(2): 351-61.
45. Lestner J, McEntee L, Johnson A, et al. Experimental Models of Short Courses of Liposomal Amphotericin B for Induction Therapy for Cryptococcal Meningitis. *Antimicrob Agents Chemother* 2017; **61**(6).
46. Bekersky I, Fielding RM, Dressler DE, Lee JW, Buell DN, Walsh TJ. Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate in humans. *Antimicrob Agents Chemother* 2002; **46**(3): 828-33.
47. Molloy SF, Kanyama C, Heyderman RS, et al. Antifungal Combinations for Treatment of Cryptococcal Meningitis in Africa. *N Engl J Med* 2018; **378**(11): 1004-17.
48. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count < or = 100 cells/microL who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010; **51**(4): 448-55.
49. Rajasingham R, Boulware DR. Reconsidering cryptococcal antigen screening in the U.S. among persons with CD4 <100 cells/mcL. *Clin Infect Dis* 2012; **55**(12): 1742-4.
50. Rajasingham R, Meya DB, Boulware DR. Integrating cryptococcal antigen screening and pre-emptive treatment into routine HIV care. *J Acquir Immune Defic Syndr* 2012; **59**(5): e85-91.
51. World Health Organization. CHOosing Interventions that are Cost Effective (WHO-CHOICE) 2015. http://www.who.int/choice/en/ and http://www.who.int/choice/costs/CER_levels/en/ (accessed 22 Oct 2015.
52. Govender N, Roy M, Mendes J, Zulu T, Chiller T, Karstaedt A. Evaluation of screening and treatment of cryptococcal antigenaemia among HIV-infected persons in Soweto, South Africa. *HIV Med* 2015.
53. Longley N, Jarvis JN, Meintjes G, et al. Cryptococcal antigen screening in patients initiating ART in South Africa: A prospective cohort study. *Clin Infect Dis* 2016; **62**(5): 581-7.
54. Letang E, Muller MC, Ntamatungiro AJ, et al. Cryptococcal Antigenemia in Immunocompromised Human Immunodeficiency Virus Patients in Rural Tanzania: A Preventable Cause of Early Mortality. *Open Forum Infect Dis* 2015; **2**(2): ofv046.
55. Desmet P, Kayembe KD, De Vroey C. The value of cryptococcal serum antigen screening among HIV-positive/AIDS patients in Kinshasa, Zaire. *AIDS* 1989; **3**(2): 77-8.
56. Lyman CA, Walsh TJ. Systemically administered antifungal agents. A review of their clinical pharmacology and therapeutic applications. *Drugs* 1992; **44**(1): 9-35.
57. Chin KG, McPherson CE, Hoffman M, Kuchta A, Mactal-Haaf C. Use of Anti-Infective Agents During Lactation: Part 2—Aminoglycosides, Macrolides, Quinolones,

Sulfonamides, Trimethoprim, Tetracyclines, Chloramphenicol, Clindamycin, and Metronidazole. *Journal of Human Lactation* 2001; **17**(1): 54-65.
58. Haddow LJ, Colebunders R, Meintjes G, et al. Cryptococcal immune reconstitution inflammatory syndrome in HIV-1-infected individuals: proposed clinical case definitions. *Lancet Infect Dis* 2010; **10**(11): 791-802.

APPENDIX

Study Sites: CRAG positive persons will be referred to one of three major centers for consideration of study participation: 1) the Infectious Diseases Institute in Kampala, Uganda; 2) Mbarara Regional Centre of Excellence; or 3) Masaka Field Station.

The Infectious Diseases Institute, Kampala

The IDI was established by the Academic Alliance for AIDS Care and Prevention in Africa (Academic Alliance), a partnership that began in 2001 between academic North American (IDSA) physicians and academic physicians at Makerere University in Kampala, Uganda, whose goal was to build the capacity to fight and control the ongoing HIV/AIDS crisis in Uganda and elsewhere in Africa. The Academic Alliance directs a training program for African physicians in the medical management of patients with HIV, including the proper use of antiretroviral therapy (ART), and has operated an HIV clinic in Kampala since 2002. The Academic Alliance, in partnership with Pangaea Global AIDS Foundation, established the two-story, 25,000 sq ft IDI Building, which was completed in September of 2004 at the Makerere University Faculty of Medicine. The IDI building houses the HIV Clinic, clinical laboratory, research labs, pharmacy, library, and educational facility. The clinic has 15 exam rooms staffed by 20 physicians, 30 nurses, and 10 counselors. Currently, approx. 350-450 patients are seen per day.

Mbarara Regional Centre of Excellence

In Mbarara, the Mbarara-JCRC clinic is adjacent to the hospital with approximately 11,000 active patients receiving HIV care. There is an onsite pharmacy, two counseling rooms, and six doctors' examination rooms, and on site facility for phlebotomy. Mbarara is located approximately 165 miles southwest of Kampala.

Both clinics have electronic medical record (EMR) systems. The clinic pharmacy dispenses a variety of medications via EMR order entry and provides free ART to eligible patients via PEPFAR and Global Fund programs. Both IDI and Mbarara research pharmacies comply with Good Clinical Practice (GCP) requirements.

Both IDI and Mbarara pharmacies have biometric locks for restricted entry as well as temperature and humidity control. The Mbarara pharmacy was upgraded in 2017.